Enantiomerically pure amines by a new method: biotransformation of oxalamic esters using the lipase from *Candida antarctica*

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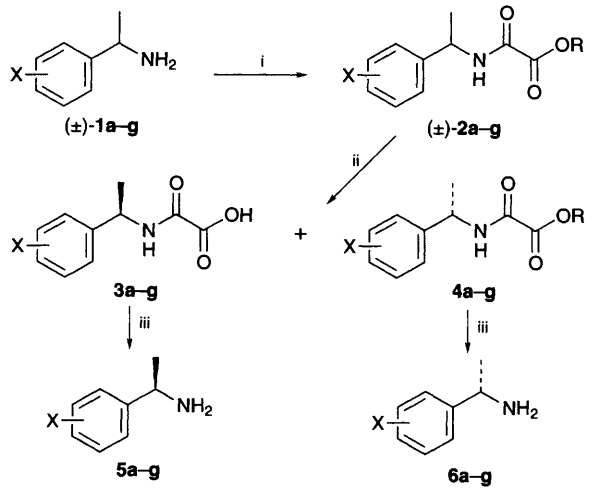
Octyl oxalamic esters of 1-phenylethylamine and substituted 1-phenylethylamines are kinetically resolved with high stereoselectivity by lipase B from *Candida antarctica*.

Many classes of compound have been successfully generated in enantiomerically pure form by biotransformation.[1] However, few methods are available for the production of enantiomerically pure amines. The Celgene Corporation has developed a process based on enzyme-catalysed transamination[2] and examples are known in which a lipase has been used to catalyse the stereoselective amidation of esters (see below).

Given that so few biotransformation methods are available in which direct manipulation of the amino function can be achieved, we turned to alternative methods in which the amine could be transformed to provide a functional group which could then be the locus of a biotransformation. It would be essential that the amine function could be regenerated both from untransformed substrate and biotransformation product. Such an approach can be regarded as 'substrate engineering', complementary to the biological approach of tailoring an enzyme to match a particular substrate type by protein engineering. Conversion of 1-phenylethylamines[1] into alkyl oxalamic esters **2** (Scheme 1) exemplifies this approach. The ester function, in principle, is susceptible to hydrolysis by lipases, esterases and proteases. The ester linkage is as close as possible to the chiral centre and both substrates and biotransformation products are readily hydrolysed to the free amine.

Through screening of a number of enzymes against the ethyl ester of the oxalamic acid of 1-phenylethylamine (**2a**, Scheme 1), lipase B from *Candida antarctica* (in the immobilised form Novozym 435 from Novo Nordisk) was identified as exhibiting the highest selectivity. Further optimisation through modification of the alkanol component of the ester revealed that selectivity increases with extension of the alkyl chain. Bioconversions with substrates **2b–g** proceeded with enantiomeric ratios of > 30 and in some cases > 100 (Table 1). Such high values for the enantiomeric ratio make it possible to obtain either biotransformation product or unhydrolysed substrate in a high state of stereochemical purity. The (*R*)-oxalamic acids **3b–g** and the (*S*)-oxalamic esters **4b–g** can be hydrolysed to the corresponding optically pure amines **5b–g** and **6b–g**, respectively.



a, X = H, R = Et; **b–g**, R = octyl; **b**, X = H; **c**, X = 2-OMe; **d**, X = 3-OMe; **e**, X = 4-OMe; **f**, X = 2-F; **g** X = 3-Br

Scheme 1 *Reagents and conditions*: i, $ClCOCO_2R$; ii, lipase B from *Candida antarctica*; iii, OH^-/H_2O

As a preparative example, ester **2b** (5 g) was incubated for three days with immobilised enzyme (Novozym 435, 0.5 g) in a mixture of a acetone (5 cm^3) and phosphate buffer (0.1 M, pH 7.0, 200 cm^3). At approximately 47% conversion the biotransformation product (**3b**, Scheme 1) was isolated and hydrolysed to the free (*R*)-amine $[\alpha]_D$ +32 (*c* 0.3, in $CHCl_3$) in 34% yield (68% of theoretical). The optical purity of the acid **3b** (95% ee) was determined by chiral HPLC (Chiralcel OD-H) of the corresponding methyl ester. In a parallel experiment in which conversion was allowed to proceed to approximately 55%, unhydrolysed substrate **4b** was hydrolysed to the free (*S*)-amine $[\alpha]_D$ −34.2 (*c* 0.3, in $CHCl_3$) of 98% ee and in unoptimised yield of 20% (40% of theoretical). All octyl oxalamic esters **4b–g** (Scheme 1) gave similar circular dichroism spectra with a negative peak at *ca.* 225 nm. Since (−)-2-methylbenzylamine has been shown unambiguously to have the (*S*)-configuration,[4] and the octyl oxalamic ester prepared from an authentic sample also showed a negative peak near 225 nm in the CD spectrum, it was concluded that hydrolysis of all of the substrates of Scheme 1 proceeded in the same sense with selectivity for hydrolysis of the (*R*)-oxalamic ester.

The method described provides a new and highly selective procedure for the production of enantiomerically pure 1-phenylethylamines. It is complementary to the procedure of lipase-catalysed acylation.[5–7] We have carried out an analysis of the selectivity of the enzyme in relation to the stereochemical course of the biotransformation and the influence of the alkanol component of the ester using the published X-ray crystal structure of the enzyme.[8] This will be described in a forthcoming paper.

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Table 1 Enantiomeric ratios for hydrolysis of oxalamic esters **2b–g**

Substrate **2**	Enantiomeric ratio[3]
b	> 100
c	> 100
d	30
e	78
f	67
g	100

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